THE CLINICAL INTERPRETATION OF THE WASSERMANN REACTION WITH SPECIAL REFERENCE TO CHOLESTERINIZED ANTIGENS.[1]

By John A. Kolmer, M.D., Dr.Ph.,
PROFESSOR OF PATHOLOGY IN THE PHILADELPHIA POLYCLINIC AND COLLEGE FOR GRADUATES IN MEDICINE,
AND
Jay F. Schamberg, A.B., M.D.,
PROFESSOR OF DISEASES OF THE SKIN IN THE PHILADELPHIA POLYCLINIC AND COLLEGE FOR GRADUATES IN MEDICINE.

(From the Laboratories of the Philadelphia Polyclinic and College for Graduates in Medicine.)

It is now well proved that the antigen in the Wassermann reaction is not necessarily biologically specific, but may be furnished by a variety of different lipoids from normal or syphilitic tissues. While it is true that extracts of a normal liver are generally inferior to extracts of syphilitic liver as antigen in this reaction, it is equally true that alcoholic extracts of normal human heart may be equal or superior to alcoholic extracts of syphilitic liver, indicating that alcohol serves to extract from heart muscle a lipoid similar in its antigenic properties at least to that found in liver which is due to the presence and activities of Treponema pallidum.

The serum of a syphilitic is characterized by the presence of an antibody-like substance, which has a great affinity for lipoids (lipodotropic), and in a mixture with them will cause the absorption or fixation of complement to a well marked degree. The old conception of the reaction which regarded the antigen as specific is erroneous; rather the nature of the antibody constitutes the specific character of the reaction because of its ability to absorb or fix complement in the presence of a lipoid. This antibody or "reagin" will, however, react better with some lipoids than with others and hence the necessity of finding that lipoid or combination of lipoidal substances which possesses most affinity or antigenic sensitiveness for the lipoidophilic antibody regardless of whether these lipoids are derived from syphilitic or normal tissues. In other words, the main principle and all that is definitely known of the syphilitic reactions is that while lipoidal extracts, as well as normal and luetic serums, may separately absorb or fix small amounts of complement, a mixture of a suitable extract and syphilitic serum is capable of fixing larger amounts of complement. Therefore, while the antigen is not biologically specific nor the antibody endowed with the property of reacting with but one lipoidal substance, the Wassermann reaction is, nevertheless, highly specific because a similar lipoidophilic antibody is found practically in two other diseases, namely, frambesia and leprosy. When these may be excluded the Wassermann reaction becomes highly specific and one of the best diagnostic aids known in modern medicine. Most efforts of late, therefore, have been made to increase the delicacy of the Wassermann reaction and yet keep it specific by attention to technical points in the hemolytic system and by attention to the tissue extract used as antigen.

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[1] Read before the College of Physicians, Philadelphia, October 8, 1914.

Since Sach's[2] originally proposed alcoholic extracts of beef heart reinforced with cholesterin as antigens in the Wassermann syphilis reaction, most observers who have employed these antigens have reported them as being quite sensitive and superior to all others. Thus McIntosh and Fildes[3] found cholesterinized heart extracts much superior to a plain extract of normal heart, to alcoholic extracts of syphilitic liver and to the lecithin-cholesterin mixture of Browning, and on account of their sensitiveness and the constancy of their qualities believe that they fulfil the requirements of a standard antigen. Walker and Swift[4] have reported equally favorable upon cholesterin heart extracts, having found them superior to alcoholic extracts of syphilitic liver, simple in preparation and of constant antigenic value. One of us (Kolmer) with Laubaugh, Casselman, and Williams[5] has been interested in this subject for several years and has made a study of cholesterinized extracts as compared with a number of other well-known antigens, particularly alcoholic extracts of syphilitic liver and acetone-insoluble lipoids. Our studies proved quite conclusively that a cholesterinized alcoholic extract of normal heart constitutes the most delicate antigen of the large number of extracts studied, acetone-insoluble lipoids coming second and proving the best non-cholesterinized extract. As compared with alcoholic extracts of syphilitic liver the cholesterinized extracts gave 29.4 per cent. stronger reactions, and what is more important yielded positive reactions in 23 per cent. of cases where the reaction with an alcoholic extract of syphilitic liver was negative. In an analysis of these cases it was found that the positive result was generally correct and but an expression of the higher antigenic sensitiveness of the cholesterinized antigens. This was true in cases yielding 50 per cent. or more inhibition of hemolysis. In a number of cases, however, the cholesterinized extracts yielded 25 per cent. or less inhibition of hemolysis with complete negative reactions with alcoholic extracts of syphilitic liver, and such cases have given us much concern as to the proper interpretation to be made of the results. Using the serums of normal persons we found 5 to 10 per cent. of these cholesterinized extracts would yield pseudoreactions of about 10 per cent. or less inhibition of hemolysis. In other words strong positive reactions with cholesterinized extracts indicate the presence of syphilis even though the reaction with alcoholic extracts of syphilitic liver were clearly negative; but weak positive reactions are more difficult to interpret and the chief object of the present communication deals with this class of reactions based upon a group of cases tested with different extracts and carefully studied from the clinical aspect.

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[2] Berl. klin. Wochnschr., 1911, xlviii, 2066.
[3] Ztschr. f. Chemotherap., Orig., 1912, pp. 1, 79.
[4] Jour. Exper. Med., 1913, xviii, 75.
[5] Archiv. Int. Med., 1913, xii, 660.

Additional reports upon cholesterinized extracts as antigens in the Wassermann reaction have been made by Field,[6] Thompson,[7] and Judd,[8] all of whom compared them with other antigens as alcoholic extracts of syphilitic liver and acetone-insoluble lipoids, and reported favorably upon the superior qualities of the cholesterinized heart extracts. Thomas and Ivy[9] secured a relatively large number of what they considered pseudo or false positive reactions and are opposed to their use.

The main purposes of this communication are fourfold:

1. To further study the antigenic value of cholesterinized alcoholic extracts of normal heart as compared with alcoholic extracts of syphilitic liver and acetone-insoluble lipoids.
2. To study with particular care those cases reacting only with cholesterinized extracts and negatively with other antigens in order to determine the specificity of the reaction with cholesterinized antigens.
3. To study the question of weak positive reactions with cholesterinized extracts and the serums of persons who deny syphilitic infection and who present no evidences of the disease.
4. To study the value of the Wassermann reaction performed with cholesterinized extracts as a control on the treatment of syphilis.

TECHNIQUE AND METHOD OF STUDY. There is general agreement among those who have employed cholesterinized antigens regarding the remarkable sensitivity and antigenic value of these extracts. Not all observers, however, have mentioned the possibility of obtaining slight degrees of inhibition of hemolysis with an occasional serum from a person who denies syphilitic infection and who presents no clinical evidences of the disease, although it is practically impossible to rule out latent hereditary syphilis with absolute certainty in all instances.

The antihemolytic activity of cholesterin is well known and unless the extracts reënforced with this substance are carefully standardized the tendency to obtain varying degrees of inhibition of hemolysis with normal serum becomes more and more in evidence. In using these extracts, therefore, the first requirement is a careful titration of their antigenic and anticomplementary values; in the Wassermann reaction we use two or three times the antigenic unit as one dose, providing that this amount is at least eight or ten times less than the anticomplementary unit.

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[6] Jour. Amer. Med. Assn., 1914, lxii, 1620.

[7] Ibid., 1458.

[8] Ibid., lxiii, 313.

[9] Ibid., lxii, 363.

If exactly one unit of complement and one unit of hemolytic amboceptor are employed in a technique the percentage of pseudoreactions with cholesterinized antigens is likely to be relatively high because of the natural antihemolytic activity of cholesterin in addition to the anticomplementary action of the alcoholic extract and patient's serum themselves. This influence may be overcome in a large percentage of cases by using at least one and a half or two units of complement or hemolytic amboceptor in conducting the reaction. It is important to remember this antihemolytic tendency of cholesterin and provide for it in our hemolytic system if antigens reënforced with cholesterin are being employed. As expected old serums, those containing appreciable amounts of thermostabile anticomplementary bodies, are more likely to show their anticomplementary condition with cholesterinized than with plain extracts.

With antigens which are carefully standardized at frequent intervals we have for several years used one and a half or two units of complement or hemolytic amboceptor in conducting the Wassermann reaction. Our usual custom is to use 0.05 c.c. (1 c.c. of 1 to 20 dilution) of fresh guinea-pig serum as a unit of complement and after titrating the hemolytic amboceptor with the complements and corpuscles used in the day's work to employ one and a half or two units of the amboceptor in the antigen titration and in the Wassermann reactions. The results of the reactions herein recorded were conducted in this manner. Even under these conditions we have found a small percentage of cases denying syphilis and showing no clinical evidences of the disease to yield slight (10 per cent. or less) inhibition of hemolysis with the cholesterinized extracts; possibly a portion of these may have been avoided by using larger amounts of complement or amboceptor; at least the importance of not using more of the antigen than is necessary and of using more than one unit of complement or amboceptor are to be emphasized.

The use of multiple units of complement or amboceptor up to two or three units does not appear to impair the delicacy of the reaction of cholesterinized extracts with syphilitic serums and lessens the effect of the antihemolytic action of the cholesterin and the tendency for false positive reactions with normal sera. It is peculiar of these reënforced extracts that when they inhibit or fix complement with syphilis antibody the degree of fixation is usually quite marked and not so readily masked by an excess of complement or hemolytic amboceptor as when plain antigens are employed.

For several years it has been our custom to use at least three different antigens with each serum in conducting the Wassermann reaction. The two main reasons for this practice are, first, to better study cholesterinized extracts as compared with plain extracts with a check upon the possibility of pseudo-reactions; and secondly, because we have reason to believe that the lipodophilic reagin or syphilis antibody in different serums concerned in the Wassermann reaction, has a different affinity for lipoidal extracts, and that these differences are independent of any changes in the antigens themselves. With the serum of active syphilis most any extract will serve to fix complement and yield a positive reaction, but when the amount of antibody is small, as in long standing or vigorously treated cases, the question of antigen and technique becomes one of considerable importance. It is in such cases that we have noted the varying degrees of reaction with different antigens of equal efficiency, and it was not uncommon to find a negative reaction with one antigen and a positive with the others. In not a few cases dependence on a single extract would have led to false negative reactions with serums of cases where treatment was being guided by the serum reactions. This is especially true of alcoholic extracts of syphilitic liver; less true of acetone-insoluble lipoids and least likely to occur with cholesterinized extracts.

It is the practice of one of us (Kolmer) to employ a cholesterinized alcoholic extract of human or beef heart, an alcoholic extract of syphilitic liver and an extract of acetone-insoluble lipoids in conducting the Wassermann reaction with each serum. As recorded by one of us in the paper previously mentioned, acetone-insoluble lipoids was found the best non-cholesterinized or plain antigen. The results of several hundred of reactions performed since then substantiate this observation. In other words, we have found cholesterinized alcoholic extracts of normal heart most sensitive; an extract of acetone-insoluble lipoids second in the order of antigenic sensitiveness and alcoholic extracts of syphilitic liver somewhat less sensitive than the other two.

Materials Used in This Study. *Antigens.* Two kinds of cholesterinized extracts were used, namely, alcoholic extracts of human and beef heart reënforced by the addition of 0.4 per cent. pure cholesterin. Human heart extract has been found superior to that of beef heart and guinea-pig heart. An alcoholic extract of syphilitic liver and suitable extracts of the acetone-insoluble lipoids from human and beef heart were used in a comparative study with the cholesterinized extracts.

Serums. In all the serums from 434 persons have been tested during the past seven months. These were mostly secured from cases in private practice and from the wards and out-patient clinics of the Polyclinic Hospital. In one series are collected 239 cases in which a history and clinical diagnosis were available; in a second series are listed 175 cases in which the diagnosis was incomplete or not given. We have excluded from this series all cases which reacted with cholesterinized extracts only because without a history they are not available for critical study.

1. A general examination of these series of cases shows that with 214 of the 414 serums the reactions were negative with all antigens including the cholesterinized extracts. In a further study of the question of false positive reactions with cholesterinized extracts we shall have occasion to refer to them in more detail.

2. With 200 serums a positive reaction was observed with one or more of the antigens. With 164 serums the reactions were positive with all three antigens but the degree of the reaction was not equal with all. Thus with 82 serums or exactly 50 per cent. the results are equal and in the remaining 50 per cent. the reactions with the different extracts varied to some degree. These are worthy of further analysis in order to study the relative sensitiveness of the different extracts.

3. Even a cursory examination of these series will suffice to show the greater antigenic sensitiveness of cholesterinized extracts; that they serve to give positive reactions with luetic serums reacting weakly or negatively with alcoholic extract of syphilitic liver and acetone-insoluble lipoids and that the tendency to produce pseudoreactions with normal serums is slight.

ANTIGENIC VALUE OF CHOLESTERINIZED EXTRACTS AS COMPARED WITH ALCOHOLIC EXTRACTS OF SYPHILITIC LIVER. 1. Of 155 serums in which a positive reaction was observed with both extracts, in 81 or 52.2 per cent. of cases the reactions were equal in both.

2. In 74 or 47.8 per cent. of cases the cholesterinized extracts gave a stronger reaction.

3. In no instance were the reactions negative with the cholesterinized and positive with the alcoholic extracts of syphilitic liver.

4. In 21 or 10.5 per cent. of 200 cases the reactions were positive with the cholesterinized extracts and negative with alcoholic extracts of syphilitic liver. These cases are worthy of further study and will be later considered in detail; here it may be stated that while the diagnoses were mostly made in the clinics and the patients were not directly under the observation of the authors, in at least 14 the history strongly suggested syphilis.

ANTIGENIC VALUE OF CHOLESTERINIZED EXTRACTS AS COMPARED WITH EXTRACTS OF ACETONE-INSOLUBLE LIPOIDS. 1. Of 169 serums in which a postive reaction was observed with both extracts, in 120 or 71 per cent. of cases the reactions were equal in both.

2. In 48 or 28.4 per cent. of cases the cholesterinized extracts yielded stronger reactions.
3. In only one case (No. 233) the reaction with a cholesterinized extract was very slightly weaker than that observed with acetone-insoluble lipoids.
4. In no instance was a reaction negative with a cholesterinized extract and positive with acetone-insoluble lipoids.
5. In 21 cases or 10.5 per cent. the reactions were positive with a cholesterinized antigen and negative with acetone-insoluble lipoids.

In the majority of these the reactions occurred with the serums of persons in whom the history and findings were strongly suggestive of syphilis.

These results, which correspond quite closely with those previously reported by one of us[10] indicate the superior antigenic sensitiveness of cholesterinized antigens as compared with other extracts which are in common use.

It may be stated, therefore, that cholesterin appears to have a more marked affinity for the lipodophilic reagin or syphilis antibody than other lipodal substances and consequently is capable of absorbing or fixing complement with this antibody to a well-marked degree. The increased sensitiveness of reënforced antigens may be due in part to the antihemolytic activities of cholesterin in a manner comparable to the increased sensitivity of serums containing thermostabile anticomplementary bodies. Or if the Wassermann reaction is interpreted as a colloidal reaction, cholesterin may be regarded as peculiarly suited for the formation of new compounds with the syphilis antibody with consequent absorption of complement to a well-marked degree. Whatever may be the explanation the fact remains as well proven that the addition of cholesterin to alcoholic extracts of normal organs and particularly of heart muscle, increases the antigenic sensitiveness of these extracts for syphilis antibody. The important question at issue, however, is in regard to the possibility of these reënforced extracts yielding false positive reactions with normal serums, that is, of its power to fix or absorb complement with some constituent of normal serum as is present to a well-marked degree in normal rabbit serum[11] and increased as a result of syphilitic infection. This is the point we have studied with particular interest.

Concerning the Specificity of the Wassermann Reaction with Cholesterinized Antigens. As just stated, most interest centres about the small percentage of cases which reacted only with the cholesterinized extracts. In practice these are the cases which give us most concern in the clinical interpretation of the reaction, especially when the degree of inhibition of hemolysis is slight. When there is 50 per cent. or more inhibition of hemolysis with a cholesterinized extract we regard the reaction as positive even though the reactions with alcoholic extracts of syphilitic liver and acetone-insoluble lipoids are clearly negative.

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[10] Kolmer, J. A., Laubaugh, E. E., Casselman, A. J., and Williams, W. W., Archiv. Int. Med., 1914, xii, 660.

[11] Kolmer, J. A., and Casselman, A. J., Jour. Med. Research, 1913, xxviii, No. 2, p. 369.

The following is a list of cases from the Polyclinic Hospital and included in one series which reacted only with cholesterinized extracts. Cases 234, 235, 236, 237, 238, and 239 were observed in the skin clinic of the Polyclinic Hospital under the eye of one of the authors.

CASE 10.—W. E., aged forty-two years. Primary optic atrophy. Had an accident to one eye in childhood. At present has Argyl-Robertson pupil. The blood reaction is as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+++	–	–

CASE 25.—E. B., aged two years. Diagnosis: enuresis; hereditary syphilis. Nothing in family history suggestive of lues. Physical examination shows occipital and cervical lymphatic glands are palpable. Has ptosis of the right eyelid. Had suspicious rash during winter.

	Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
July 27, 1914	++–+++	–	–
July 31, 1914	+++	–	–

CASE 35.—S. B., aged twenty-one years. Diagnosis: tumor of breast. Nothing in history suggestive of syphilis. Patient has a mass in the breast that appeared to be tuberculous. The blood reaction was as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	–	–

CASE 55.—M. C., aged sixty-eight years. Diagnosis: carcinoma of liver or cirrhosis of liver. Patient had failure of health four years previous to admission. Was married, had three children; all died in early life. One was stillborn; one died at age of one and a half years and one died at age of fifteen years. Causes not stated. Father had paralysis before he died. The blood reaction was as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
++	–	–

CASE 85.—H. S., aged two years. Infantile hemiplegia; nothing in history suggesting lues; present sickness came on with chills, fever, and convulsions, followed by a paralysis of left arm and leg, also could not speak. Regained power of speech, but cannot walk. The blood reaction was as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	–	–

CASE 106.—H. R., aged four years. Diagnosis: acute cardiac dilatation and chorea. Nothing in family history suggestive of lues. Mother had no miscarriages; no stillbirths. Physical examination shows that all the superficial lymphatic glands are enlarged and not tender. Present sickness began with a chill, fever, and pain in the wrists. The blood reaction was as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble. lipoids.
+	–	–

CASE 127.—T. M., aged fifty-eight years. Primary diagnosis: locomotor ataxia; secondary diagnosis: gastritis. Patient denied any luetic infection. Nothing in history suggesting syphilis. (There was quite a dispute as to the diagnosis in this case.) Knee-jerks were quite suggestive of gastric crises. Tabes was the diagnosis severally suggested. The blood reaction is as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	–	–

CASE 134.—A. M., aged fifty-six years. Primary diagnosis: acute dementia; secondary diagnosis, neurasthenia. Patient had eleven children, all dead but three; causes not stated. Patient complains of indefinite pain in all parts of her body. The blood reaction was as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
++	–	–

CASE 168.—S. G., aged forty-three years. Primary diagnosis: osteitis (luetic?). The blood reaction was as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	–	–

CASE 174.—R. G., aged nineteen years. Nothing whatsoever in history suggestive of lues. Patient suffering from nausea, loss

of weight. Patient was put on protiodide on July 16, 1914, but no data as to results of the treatment. Girl has suspicious patches on tongue. The reaction of the blood was as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
++++	−	−

CASE 200.—E. H., aged twenty years. Diagnosis: psychosis. Nothing in history suggesting syphilis. Patient became melancholy for no apparent cause. Refused to talk, walk, or eat. Was afraid to be touched and refused all assistance. Was in the hospital but three days. A complete family history was not obtained. Right-sided paralysis. The blood reaction was as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	−	−

CASE 201.—B. D., aged forty-two years. Diagnosis: chronic gastritis; arteriosclerosis. Pain in epigastrium for past year. Nausea and sometimes vomiting. History of chancre; enlarged epitrochlear glands. The blood reaction was as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble. lipoids.
++++	−	−

CASE 205.—M. T., aged two months. Diagnosis: gastroenteritis and malnutrition. Child healthy at birth. Bottle fed. First birth. No history of lues. Mother states that child had blebs on soles of feet and palms of hands at birth. No skin eruption. At time patient was admitted there was a distinct shedding of the skin on the palmar and plantar surfaces. The blood reaction was as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
++++	−	−

CASE 207.—W. S., aged forty-five years. Diagnosis: carcinoma of stomach. Nothing whatsoever suggestive of syphilis in the history; however, there was no negative statement as to luetic infection. Pain in epigastrium. The blood reaction was as follows:

	Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
May 10, 1914	±	−	−
May 12, 1914	±	−	−

CASE 214.—T. K., aged thirty-six years. Diagnosis: acute neuritis. Nothing in history or physical examination suggestive of syphilis. This patient was put on KI gr. VII t.i.d. and on the

second day after taking medicine patient developed a severe coryza which quickly cleared up upon withdrawal of the drug. Pain in legs about course of anterior and posterior tibial nerves; weakness after standing. The blood reaction is as follows:

Cholesterin-ized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	–	–

Case 234.—I. A., aged ten years. Lues hereditaria. Patient had extensive ulcerative and vegetative lesions on the right arm and hand. His serum reaction on was as follows:

Cholesterin-ized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	–	–

Under appropriate specific improvement a striking improvement in the eruption was brought about.

Case 235.—F. McL., aged fifty years. Was treated several years ago for a squamous syphilide of the palm of the hand. Had been under internal treatment for some years. On April 10, 1914, his reaction was as follows:

Cholesterin-ized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	–	–

Case 236.—Mrs. K. Developed an initial lesion on the lip in April, 1913. Husband was being treated for lues. This patient had had salvarsan and mercurial inunctions. Her serum reactions were as follows:

	Cholesterin-ized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
June 29, 1914	+	– (D. H.)	–
September 16, 1914	++	–	+

Case 237.—O. R., aged fifty-nine years. Slight treatment at the dispensary for pruritus of the genitalia. It was noticed that he had luetic scars on the forehead and blood was taken for a Wassermann reaction.

Cholesterin-ized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	–	–

Case 238.—P. McG., aged forty-four years, presented himself with an eruption upon the lower lip of suspicious character. Duration two years. His Wassermann reaction yielded the following result:

Cholesterin-ized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+++	–	–

Case 239.—D. M., aged thirty years. Patient has been under observation with a rebellious and severe sycosis vulgaris involving the cheeks, chin, and lip. There is no history of lues and the patient's general health is good. On April 2, 1914 his serum reacted as follows:

Cholesterinized beef heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble. lipoids.
+	– (D. H.)	– (D. H.)

In this case we are not persuaded that the reaction indicates lues; the possibility of a latent lues hereditaria, however, can never be quite eliminated, and the interpretation of the reaction in this patient must remain open.

The list above given representing ambulatory hospital patients might readily contain inaccuracies of diagnosis such as not infrequently occur in dispensary practice. It will, perhaps, be more valuable therefore to study a series of patients in private practice. Below is appended a list of 20 such patients who have been under the observation of one of the authors for from one to six years. All of these patients are luetic subjects, and multiple serological tests have been made on practically all. These patients have shown at times a positive Wassermann reaction with the alcoholic extracts of syphilitic liver and an extract of acetone-insoluble lipoids, but have at other times been negative with these and positive only with the cholesterinized antigens. The persistence of negative Wassermanns in these cases would have suggested an extinction of the spirochetic infection and consequent cessation of treatment, had not the cholesterinized antigen been employed. Most of these patients were in the secondary and tertiary stage. Some of these patients after exhibiting positive reactions only with the cholesterinized antigens later gave frank positives with an alcoholic extract of syphilitic liver. One patient with a four-year-old infection had had several negative Wassermanns reported by a competent serologist. He presented himself for treatment with an annular nodular eruption upon the side of the chin; he was much surprised and shocked when he was informed that the patch was syphilitic for he was hopeful that he had been cured. A blood test was again made and was negative with alcoholic extract of syphilitic liver, but weakly positive with the extract of syphilitic liver and strongly positive with the cholesterinized extracts, and intravenous injection of salvarsan effected a rapid disappearance of the patch.

A brief history of these cases is appended:

Case 1.—J. W., aged thirty-two years. Infection in 1901. Treated by his physician with internal remedies for two years. He has since July, 1913, received ten intravenous injections of salvarsan and neosalvarsan and a course of gray-oil injections and is entirely free of any manifestations of disease. The record of his blood test is as follows:

	Cholesterin-ized beef heart.	Cholesterin-ized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
December 30, 1913	±	±	−	−
February 11, 1914	+++	....	++	±
April 27, 1914	+++	−	−(D. H.)	−
June 13, 1914	−	−	−(D. H.)	+
September 6, 1914	+++	....	±	

It will here be seen that when the patient came first under observation the serum test with the alcoholic extract of syphilitic liver and acetone-insoluble lipoids was negative, with positive reactions only with the cholesterinized extracts. As indicating that the latter tests meant spirochetic activity, the tests later became positive with the other antigens.

Case 2.—W. S., aged fifty-five years. Infection dates back to early manhood. Patient has arteriosclerosis. Wife treated ten years ago for placques on tongue. The record of the serum test is as follows:

	Cholesterin-ized beef heart.	Cholesterin-ized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
March 4, 1911	....	....	Weak +	Weak +
September 26, 1911	....	....	++	++
November 24, 1911	....	....	−	±
May 23, 1912	....	....	+	+
Octo ber23, 1912	....	....	+	++
March 14, 1913	....	....	−	−
May 8, 1913	....	....	−	−
June 12, 1913	+	+	−	±
November 13, 1913	+	±	−	−
June 24, 1914	+++	....	−	−

This patient has had seven intravenous injections of salvarsan and neosalvarsan, in addition he received mercurial injections. His health has been much improved. The five tests above indicated that are negative with the alcoholic extract of syphilitic liver do not persuade us that infection is eradicated.

Case 3.—J. K., aged thirty-three years. Infection dates back five years. Had an entirely inadequate treatment during the secondary period. Patient came under the care of one of the authors in December, 1912. A blood test taken about three weeks after the cessation of mercurial inunctions was negative. The serum reactions are herewith appended:

	Cholesterin-ized beef heart.	Cholesterin-ized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
December 2, 1912	....	....	−	
September 27, 1913	++++	++++	++++	++++
April 7, 1914	+++	−	−	−
June 27, 1914	++	....	+	+

The first test when the patient came under our observation was made by a very competent serologist in this city; it doubtless had been influenced by the preceding treatment. The patient received while under our care thirteen gray-oil injections, five intravenous injections of neosalvarsan and other treatment.

Case 4.—S. H., aged thirty-two years. Had a suspicious sore three years ago, the nature of which was not determined by his physician at that time. The patient came under observation in the autumn of 1913, at which time he had numerous ulcerative lesions on the thighs and legs. His blood reactions were as follows:

	Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
October 21, 1913	++++	++++	++++	++++
March 24, 1914	+	+	–	–
June 2, 1914	++	++	–	–

The ulcerations healed up under two intravenous injections of neosalvarsan. The patient received in all eight intravenous injections of neosalvarsan and salvarsan and eight gray-oil injections. He is at present free of symptoms, but is still under treatment.

Case 5.—F. G., aged twenty-six years. Had an initial lesion followed by atypical secondaries in June, 1912. Received mercurial inunctions and other treatment.

	Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
March 5, 1913	....	....	–	–
September 13, 1913	++++	++++	+++	+++
March 10, 1914	–	....	–	–
May 5, 1914	–	....	–	–
July 19, 1914	++	....	–	–

On September 19, 1913, he received 0.45 gram salvarsan intravenously, a second dose on September 28, 1913, and a third on October 3, 1913; this was followed by a course of gray-oil injections. Salvarsan intravenously on July 15, 1914 and July 22, 1914. The patient is free of symptoms.

Case 6.—S. F., aged five years. Father treated for affection some years ago; child has mild degree of luetic cachexia; no florid symptoms. The blood reactions are as follows:

	Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
February 2, 1911	....	....	–	+ faint
May 23, 1911	....	....	–	
September 7, 1911	....	....	±	
September 27, 1911, while taking KI and Hg. bichlor.	....	....	+	+

	Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
December 2, 1912	±	+	–	–
September 16, 1913	–	–	–	–
November 14, 1913	–	–	–	–
February 11, 1914	–	–	–	–

On May 21, 1914, the luetin test was slightly positive.

From October 9, 1911, to September 18, 1914, this patient received nineteen gluteal injections, each containing 100 milligrams of salvarsan in oil.

CASE 7.—Miss H., aged thirty years; luetic infection in 1909. No active symptoms during past three years. Had a positive Wassermann before presenting herself for treatment. Received intravenous injections of salvarsan on March 27, 1913, April 7, 1913, October 27, 1913, and November 10, 1913 in addition to other treatment. On October 1, 1913, her serum test was:

Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	+	–	–

The patient is free of symptoms of lues.

CASE 8.—Mrs. S., wife of a patient (Case 2). Had persistent placques on tongue over ten years ago.

	Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
May 19, 1913	....	....	–	±
November 11, 1913	+	++	–	–

The patient received salvarsan and gray-oil injections, after which her reaction became negative with all antigens.

	Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
April 14, 1914	–	–	–	–

CASE 9.—W. C., aged thirty-six years. Initial lesion four years ago. Treated by his physician with pills for two years. He then had several negative Wassermann reactions. In the late Spring of 1914 he developed an annulonodular patch upon the side of the chin which was readily diagnosed as a syphilide. His blood at this time gave the following reaction:

		Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
May 21, 1914	....	+++	–	–

An intravenous injection of salvarsan effected a disappearance of the patch; this was followed by two other injections; later the reaction with the alcoholic extract of syphilitic liver became positive.

		Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
June 27, 1914	....	++	+	+
September 18, 1914	....	++++	+	++++

The patient is now free of symptoms.

CASE 10.—P. V., aged fifty-five years. Infected at age of twenty-eight. Very little early treatment. Later extensive ulcerations of leg. Treatment by mouth then given for three years by patient's physician. This patient had some neuritic pains in arm. On April 27, 1914, his blood showed the following:

Cholesterin-ized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	–	–

He was then given three intravenous injections of salvarsan; two months later the reactions was as follows:

	Cholesterin-ized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
June 24, 1914	++++	–	–

Since this time further treatment has been instituted.

CASE 11.—F. S., aged forty years. Infection at age of twenty-one years. Insufficient early treatment. The patient has premature arterial fibrosis. He has had five intravenous injections of salvarsan, gray-oil, and cacodylate of mercury injections and other treatment. He has had negative serum reactions on a number of occasions only to be followed by a swing to a positive reaction. The reactions for the past two years are herewith appended:

	Cholesterin-ized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
February 19, 1913	....	–	–
April 4, 1913	....	–	–
June 17, 1913	+	–	–
November 11, 1913	±	–	–
February 1, 1914	+	–	–
May 4, 1914	–	–	–
July 11, 1914	+++	–	–
September 28, 1914	–	–	–

It will be seen that the alcoholic extract of syphilitic liver and the acetone-insoluble lipoids have given negative reactions for over a year, but with the cholesterinized antigens the serum has given positive reactions from time to time. In support of the contention that the latter indicates that the spirochetal infections is not extinguished may be cited the fact that on March 20, 1914, after the Wassermann had been negative for almost a year, there was a strongly positive luetin reaction and that on May 7, 1914, the luetin reaction was again positive, although this time the lesion did not go on to pustulation.

CASE 12.—H. G., aged forty-two years; infected over twenty years ago. Inadequate early treatment. Patient has arterial changes, loss of sexual power, and certain other symptoms suggestive of incipient paresis. The complement-fixation test on September 14, 1914, was as follows:

	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
....	++ to +++	–	–

Several intravenous injections of salvarsan have greatly improved the patient's condition.

Case 13.—A. F., aged thirty-eight years; infection seven years ago. Has been thoroughly treated by salvarsan and mercurial injections. On June 4, 1913 the serum reaction was:

Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
±	+	–	–

Case 14.—J. K., a college student, aged twenty-two years; infected six years ago. On October 12, 1912, the complement-fixation test was as follows:

Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
++++	++++

After treatment the reaction became negative on March 20, 1913. On June 6, 1913, the following reaction was obtained:

Cholesterinized guinea-pig heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	+	–	–

The patient did not return for treatment; in the Autumn of 1913 he entered a Western University; his blood examined at that time was frankly positive with the alcoholic extract of syphilitic liver, according to a communication received from one of the professors in this institution.

Case 15.—Dr. X., aged thirty years. Digital chancre three years ago. Intravenous injections of salvarsan and other treatment. On February 28, 1912, the serum reaction was:

	Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
February 28, 1912	....	....	+	+
May 9, 1912	....	....	+++	+++
June 25, 1912	....	....	±	+
October 24, 1912	....	....	Weak +	Weak +
December 8, 1913	....	....	–	±
February 24, 1913	....	....	Weak +	Weak +
June 30, 1913	±	–	–	–
October 14, 1913	+++	++	+	±
January 4, 1914	–	–	–	–
February 11, 1914	±	–	–	–
May 4, 1914	....	–	–	–

Treatment was continued as long as positive reactions were obtained.

CASE 16.—Mrs. A., aged forty-five years. Infection fifteen years ago. Had tertiary ulcerative lesions on elbow when she fitst came under observation. The patient was treated by salvarsan intravenously and mercury iodides by mouth.

	Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
January 19, 1914	++	+ to ++	–	–

The patient lived out of town and did not continue the treatment after the above test was made. The reaction, however, indicates the need of further treatment.

CASE 17.—A. H., aged fifty-three years. Infection sixteen years ago. Patient presented himself with a tertiary ulceration of cheek. Intravenous injections of salvarsan and internal treatment.

	Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
November 11, 1913	+	–	–	–

The patient has not returned for treatment since the above test was made.

CASE 18.—Mrs. W., aged thirty years. Husband treated for lues. Anemia and failure of health. This patient has been treated with salvarsan and mercury. The serum reactions are as follows:

	Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
November 2, 1911	....	....	±	+
November 15, 1912	....	....	±	Weak +
November 25, 1913	± to +	–	–	–
April 8, 1914	....	....	–	–

CASE 19.—M. G., aged forty-eight years. Infected twenty years ago; early treatment inadequate. Recently treated by salvarsan and mercury.

	Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
October 22, 1913	–	+ to ++	–	–
June 24, 1914	–	....	–	–

CASE 20.—Mrs. C., aged forty-eight years. No history of lues. Has had several years recurrent sores in mouth. The serum reaction was as follows:

Cholesterinized beef heart.	Cholesterinized human heart.	Alcoholic extract of syphilitic liver.	Acetone-insoluble lipoids.
+	+	–	–

A gluteal injection of salvarsan was followed by marked improvement.

Of these 41 *cases, no less than* 20 *were known to be definitely syphilitic and in at least* 14 *others the diagnosis of lues was most probable. In other words, in* 34 *or about* 83 *per cent. of these cases, the positive reactions with the cholesterinized extract were in all probability correct and an expression of continued spirochetal activity. What is of great importance is the fact that if these extracts had not been used either a diagnosis of syphilis would not have been made or treatment would have been interrupted before spirochetal activity had ceased.*

In 7 cases, however, the reactions were negative with an alcoholic extract of syphilitic liver and acetone-insoluble lipoids, but weakly positive with cholesterinized heart extracts. In none of these cases, as far as the available records are concerned, was there clinical evidence of syphilis, although the dispensary physicians did not record careful family histories with the possibility of congenital syphilis in mind.

These cases are typical of the difficulties of interpretation of the Wassermann reaction with cholesterinized extracts. All weakly positive reactions with these antigens cannot be disregarded, for there is a possibility that such reactions are true positive reactions. Especially is this true of cases of syphilis under treatment and also of cases of latent tertiary syphilis.

Not infrequently serums are encountered which react strongly positive with cholesterinized extracts and negatively with alcoholic extracts of syphilitic liver and acetone-insoluble lipoids. We have found that these are almost invariably from luetic persons and have learned by experience to interpret them as positive reactions. The chief difficulty is with serums which react weakly with cholesterinized extracts and negatively with plain extracts. According to our experience most of these are from luetic persons and the reactions are to be regarded as positive; but this at the present time cannot be claimed for all such reactions so that the result must be interpreted in the light of the history of symptoms and a physical examination. If there is a reasonable suspicion of syphilis we have found it well to administer antiluetic treatment, as in several instances persons yielding these doubtful reactions have shown marked improvement in their clinical condition followed in some instances by a stronger Wassermann reaction.

This is called a "provocative" reaction and is similar to the clinical Jarisch-Herxheimer reaction. We may state that an extended experience with cholesterinized extracts has steadily increased our confidence in their value; we desire, however, to make a reservation concerning the possibility of cholesterinized extracts delaying hemolysis or even yielding weakly positive reactions with the serums of persons who are apparently nonsyphilitic.

In the presence of such a reaction, namely, a weak positive with a cholesterinized extract and clear negative with alcoholic extract of syphilitic liver and acetone-insoluble lipoids, the result should be interpreted according to the history and clinical symptoms of the patient. If the history is suggestive of lues the reaction should be in the interests of the patient interpreted as a positive; if the patient is a luetic subject under treatment, further treatment is indicated. In a number of instances of such reactions in persons in whom infection was denied, marked clinical improvement followed the administration of salvarsan and mercury.

III. Concerning the Question of Pseudoreactions with Cholesterinized Antigens. In a previous paper one of us had directed attention to the possibility of obtaining weakly positive reactions of 10 per cent. or less inhibition of hemolysis with 5 to 10 per cent. normal serums. At that time we were using with the cholesterinized extracts a hemolytic system which did not permit of a sufficient excess of complement or hemolytic amboceptor to overcome the increased antihemolytic action of these extracts due to the presence of so much cholesterin. As already pointed out it is necessary to carefully standardize the antigen and to use no more than necessary and to use more than a single unit of complement or hemolytic amboceptor.

Of the serums of 434 persons submitted for Wassermann reaction and included in this study, 214 reacted negatively with all antigens including the cholesterinized extracts. It is possible that some of these were luetic but the number of negative reactions with cholesterinized antigens and the serums of persons who were apparently healthy or showing no signs of the disease and with a negative history of infection is such as tends to minimize the likelihood of false positive reactions with these extracts.

In 7 out of 41 reacting only with cholesterinized extracts there was no adequate evidence of syphilis, but as already stated there was no rigid inquiry made as to the family history by the dispensary physicians who sent the blood for diagnosis, and the possibility of a latent infection cannot be absolutely set aside. We do not feel justified in regarding these cases as luetic but a final judgement as to the interpretation of such reactions must be left to the future.

IV. The Use of Cholesterinized Antigens in the Wassermann Reaction as a Therapeutic Guide. We hold that the alcoholic extract of syphilitic liver, the usual antigen employed in the Wassermann reaction, is not sufficiently sensitive to detect minute amounts of "reagin" in the blood. Many reactions are reported as negative with this antigen in which the use of cholesterinized antigens gives positive reactions. That in the vast majority of cases these latter positives indicate spirochetic activity, we believe is proven by the accompanying case reports. In patients known to have had syphilis, positives with cholesterinized antigens definitely demonstrate that the patient is not cured.

The interpretation of weak positive reactions with the cholesterinized antigens and negative with luetic liver extract will depend somewhat upon collateral evidence. Where the patient is known to have had syphilis, weak positives with cholesterinized extracts, to our minds, indicate the persistence of spirochetic activity. This opinion is based (1) upon the fact that some of these patients, particularly when treatment is interrupted, will later show frank positives with other antigens; (2) that some patients with obvious luetic lesions present such serum reactions; (3) that some of our patients with such reactions have given positive luetin reactions; (4) that certain patients with such reactions, exhibiting no definite syphilitic manifestations, but such symptoms as anemia, failure of health, ocular or nervous diseases of undetermined character, respond often in a surprising manner to specific treatment, and (5) finally, that the persistence of specific treatment in patients with weak positives with cholesterinized extracts, will often extinguish such reactions. One of the authors has had 25 luetic patients in whom he was able to secure a negative reaction with all of the antigens employed.

It is obvious that when the "reagin" in the blood has been reduced to a point where it is no longer discoverable by the use of luetic liver extracts but only by more sensitive antigens, the patient is in the best position to have his infection eradicated, provided treatment is not suspended. This is a practical consideration upon which we wish to place emphasis.

A luetic person under treatment will react negatively with alcoholic extracts of syphilitic liver and acetone-insoluble lipoids for some time before the reaction becomes negative with cholesterinized extracts. In other words, it requires more treatment to extinguish a reaction with reënforced antigens than with plain extracts. We have several reasons for believing that treatment should be continued as long as the serum reaction with cholesterinized antigens remains positive to the slightest degree and a number of our cases in private practice have proven that with sufficient treatment the reaction may be rendered negative with all antigens. We have had several "Wassermann fast" patients whose serum reactions cannot be altered by persistent or rigorous treatment. One patient with an old lues has received 19 injections of salvarsan and neosalvarsan in addition to other treatment and still has a ++++ reaction. Such cases usually react positively with all antigens but rather more strongly with the cholesterinized than the plain extracts.

Discussion. Not infrequently the statement is made that the diagnosis of syphilis should never be made in the laboratory. In not a smaller proportion of cases, however, especially cases of latent and hereditary syphilis the diagnosis cannot be made otherwise. Indeed, the Wassermann reaction has its chief usefulness in this class of cases. Persons may be sincere in denying knowledge of infection and the symptoms may be of such an indefinite character as to make a clinical diagnosis impossible. It is generally conceded that the Wassermann reaction serves as an important guide to treatment; this is based upon the assumption that a positive reaction indicates the presence of living spirochetes within the tissues, although these may not be sufficiently active or numerous or so situated as to produce obvious symptoms.

With these considerations in mind it may be granted that the reaction should be made as delicate as is possible within the limits of specificity. In the diagnosis of syphilis by the Wassermann reaction every possible precaution should be taken in the technique to avoid error. Such a diagnosis, pregnant as it is with the infliction of mental suffering and at times domestic unhappiness, should be made only after the establishing of conclusive laboratory evidence or after a mature consideration of conjoint laboratory and clinical data. In doubtful cases it is better to err on the side of announcing the reaction as negative. The physician submitting the blood for diagnosis should, however, receive a supplementary statement to the effect that the absence of syphilis cannot be conclusively demonstrated, and, that any suspicious symptoms should be submitted to the therapeutic test.

In using the reaction as a guide to treatment the technique should be as delicate as is compatible with specificity in order to detect the minutest amounts of antibody present. The persistence of ever so few living spirochetes in the body may later give rise to serious manifestations.

According to our experience cholesterinized antigens are the most delicate with which we are acquainted. A negative reaction with a good cholesterinized extract as antigen excludes syphilis more definitely than is possible with any other extract. Furthermore, this antigen will detect a larger proportion of latent luetic infections than any other antigen. Cholesterinized antigens necessitate more prolonged and more thorough treatment of syphilitics than when plain antigens are used in the technique. All of these are advantages, as they tend to more accuracy in diagnosis and greater throughness in treatment.

It is to be emphasized, however, that this delicacy renders these antigens dangerous in inexperienced hands. They must be frequently and carefully tested and standardized and used with a hemolytic system embracing more than a single unit of complement and amboceptor. After an experience of a number of years with all of the better-known extracts, we would hesitate to report upon a Wassermann reaction unless at least one satisfactory cholesterinized extract was used as an antigen in the test.

CONCLUSIONS. 1. Cholesterinized alcoholic extracts of normal heart constitute more sensitive antigen in the Wassermann reaction than alcoholic extracts of syphilitic liver and extracts of acetone-insoluble lipoids. The latter extracts are superior to alcoholic extracts of syphilitic liver and rank second to the cholesterinized extracts in antigenic sensitiveness.

2. Of 41 cases reacting only with cholesterinized antigens no less than 20 were known definitely to be syphilitic and in at least 14 the diagnosis of lues was most probable. In other words, in 34 or about 83 per cent. of the cases the positive reactions with the cholesterinized extracts were in all probability correct and an expression of continued spirochetal activity, even though the reactions with alcoholic extracts of syphilitic liver and acetone-insoluble lipoids were negative. What is of great importance is the fact that if these extracts had not been used either a diagnosis of syphilis would not have been made or treatment would have been interrupted before spirochetal activity had ceased.

3. We are of the opinion that cholesterinized extracts are best employed in conjunction with non-cholesterinized extracts in performing the Wassermann reaction and very slight positive reactions with the cholesterinized extracts should be interpreted in conjunction with a clinical study of the patient. With a person known to be syphilitic these slight reactions are to be interpreted as positive and as an indication for further treatment.

4. The large number of negative reactions with cholesterinized antigens and the serums of persons in good health or those known to be suffering from non-luetic conditions is evidence that false positive or pseudoreactions with these extracts are rare.

5. In using cholesterinized antigens it is not advisable to work with single units of complement and amboceptor because of the well-known antihemolytic properties of cholesterin, and the corresponding increased likelihood of obtaining false reaction.

6. Cholesterinized alcoholic extracts of heart are easily prepared and are quite stable. It is advisable, however, to retitrate them at frequent intervals as is usual with other antigens.

7. Cholesterinized alcoholic extracts of human heart are probably better than those of beef and guinea-pig heart. The latter extracts are particularly prone to become anticomplementary.

8. Cholesterinized extracts constitute the best antigens when the treatment of syphilis is being guided by the Wassermann reaction reaction, because they are the most delicate and serve to indicate spirochetal activity after the reactions with alcoholic extracts of syphilitic liver have become negative. It requires more treatment to extinguish the Wassermann reaction when cholesterinized extracts are employed. This result, however, can be achieved, and when the reactions so performed continue to be negative, the test constitutes the best evidence of the cure of syphilis that we possess.